PATENT COOPERATION TREATY 09/622101

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 12.05.00
Applicant's or agent's file reference B 3270 PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/00945	12/02/1999	13/02/1998

Applicant
MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSC

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
B 3270 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/00945	12/02/1999	13/02/1998
International Patent Classification (IPC) or national classification and IPC C12P21/00		
Applicant MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSC		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/09/1999	12.05.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Marie, A Telephone No. +49 89 2399 8413

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/00945

I. Basis of th report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-64	as originally filed

Claims, No.:

1-38	as received on	17/04/2000	with letter of	17/04/2000

Drawings, sheets:

1/7-7/7	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-22,24-38
	No:	Claims	23-23
Inventive step (IS)	Yes:	Claims	1-22,24-31,36,38
	No:	Claims	23-25,32-35,37
Industrial applicability (IA)	Yes:	Claims	1-38
	No:	Claims	

2. Citations and explanations

see separate sheet

1. As far as the priority right can be acknowledged, **Biochemistry, 1998, 37, 3677-3686** (**D1**) and **PNAS, 1998, 95, 14045-14050** (**D2**) cannot be taken into consideration, since they have been published after the priority date.

2. The problem underlying the failure of AZT is described in **Nature Medicine, 1997, 3/8, 836-837** (**D3**) and/or **Nature Structural Biology, 1997, 4/8, 601-604** (**D4**). In particular, the importance of the P-loop and the LID region is emphasised. Furthermore, the movement of the P-loop resulting in a 200-fold reduced phosphorylation rate of AZTMP in mentioned in **D4** on page 602.
D4 further suggests that 2 choices could be made to avoid said reduced phosphorylation rate and accumulation of toxic AZTMP, one of them is to modify TmpK (page 603).
However, none of these documents describes or suggests the solutions given in the present application. Therefore, basically the subject-matter of the present claims has to be considered novel (Article 33.2 PCT) and inventive (Article 33.3 PCT) over the cited prior art as fas as said claims are related to a polypeptide modified according to claim 1.

3.1 However, claim 23 embraces a composition containing only a non-modified nucleotide kinase, i.e. the item (a). Indeed, items (b)-(c) are either introduced with the adverb "...**optionally**..." or the preposition "...**or**...". Insofar they have no limitation affect and must not be present in the claimed composition.
Since, TmpK is described in both **D3** and **D4**, claims 23-25 cannot be considered as novel and/or inventive (Articles 33.2 and 33.3 PCT). This also applies to claim 37

3.2 An objection under Article 33.3 PCT can be raised against claims 32-35, 37 which also embrace *inter alia* the use of "...*Use of a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog...for the preparation of a pharmaceutical composition ...*", since said use is directly derivable from the known biological activity of said enzyme.

4. The formulation of claim 1 is not so clear (Article 6 PCT) because of the expression "...polypeptide *having or having an enhanced* kinase activity...".

5. Claims 32, 35 may give rise under certain patent laws to objections, since they can be considered as methods of treatment of the human body.

PCT/EP99/00945
Max-Planck-Gesellschaft zur
Förderung der Wissenschaften e.V.
Our Ref.: B 3270 PCT

New set of claims

1. A method for the production of a polypeptide having or having enhanced kinase activity for a nucleoside or nucleotide analog, said method comprising substituting, adding or deleting at least one amino acid of a protein having nucleoside or nucleotide kinase activity at a position in the protein where:
   (a) the amino acid is at position X_2 and/or X_3 in the consensus sequence $GX_1X_2X_3X_4GK$ of the P-loop;
   (b) the amino acid is in the LID region; and/or
   (c) the amino acid is at position 105 in the amino acid sequence of human thymidylate kinase or at a corresponding position in a protein having nucleoside or nucleotide kinase activity.

2. The method of claim 1, wherein said nucleoside is adenosine, cytidine, guanosine, thymidine or uridine or based on any of these.

3. The method of claim 1 or 2, wherein said nucleotide is a nucleoside monophosphate.

4. The method of claim 3, wherein said nucleoside monophosphate is thymidylate.

5. The method of any one of claims 1 to 4, wherein said protein is derived from a eukaryotic or prokaryotic organism.

6. The method of claim 5, wherein said organism is human or a yeast.

7. The method of any one of claims 1 to 6, wherein said protein comprises the amino acid sequence of any one of SEQ ID NOS: 1 to 13 or a fragment thereof.

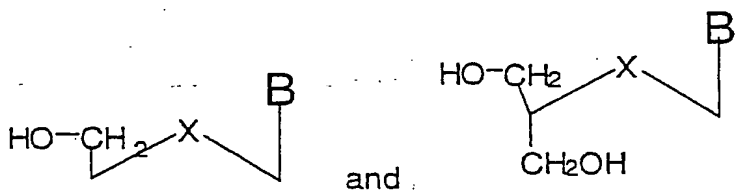
8. The method of any one of claims 1 to 7, wherein said amino acid which is substituted or added in (a) is glutamic acid, glycine or lysine and the amino acid substituted in (c) is tyrosine.

9. The method of any one of claims 1 to 8, wherein said amino acid which is substituted or added in (b) is a basic amino acid, preferably arginine.

10. The method of any one of claims 1 to 9 where the LID has the consensus sequence R/KXXXXXERYEXXXXQ.

11. The method of any one of claims 1 to 10, wherein said polypeptide exhibits kinase activity for a nucleoside or nucleotide analog which is higher than that of the/a corresponding wild type eukaryotic, preferably human enzyme.

12. The method of any one of claims 1 to 11, wherein said nucleoside analog is AZT, d4T or has the following structure:



wherein B is any nucleobase or analog thereof, and X is O, CH_2, NH or S.

13. The method of any one of claims 1 to 12, wherein the amino acid substitution(s) result in the P-loop and/or in the LID region of a bacterial nucleoside or nucleotide kinase, preferably those of the TmpK of E.coli.

14. A polynucleotide encoding the polypeptide obtainable by the method of any one of claims 1 to 13.

15. A vector containing the polynucleotide of claim 14.

16. The vector of claim 15, wherein the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells.

17. The vector of claim 15 or 16, which is a gene transfer or a gene targeting vector.

18. A host cell genetically engineered with the vector of any one of claims 15 to 17.

19. A method for producing a polypeptide having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog comprising
    (a) culturing the host cell of claim 18, and
    (b) recovering said polypeptide from the culture.

20. A method for producing cells capable of expressing a polypeptide having nucleoside or nucleotide kinase activity for nucleoside or nucleotide analogs comprising genetically engineering cells with the polynucleotide of claim 14, or with the vector of any one of claims 15 to 17.

21. A polypeptide having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog encoded by polynucleotide of claim 14, obtainable by the method of any one of claims 1 to 13 or 19 or from cells produced by the method of claim 20 or comprising a biologically active fragment of any of these.

22. An antibody specifically recognizing the polypeptide of claim 21.

23. A composition comprising
    (a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide; or

(b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17;

(c) optionally a nucleoside or nucleotide analog; and

(d) optionally a pharmaceutically acceptable carrier.

24. The composition of claim 23, wherein said protein is a bacterial nucleoside or nucleotide kinase.

25. The composition of claim 23 or 24, wherein said protein is a bacterial TmpK.

26. The composition of any one of claims 23 to 25, wherein said protein has at least the P-loop and/or the LID region of E.coli TmpK.

27. The composition of claim 25 or 26, wherein said TmpK comprises the amino acid sequence shown in SEQ ID NO: 4 or a biologically active fragment thereof.

28. A kit comprising the polynucleotide of claim 14, the vector of any one of claims 15 to 17, the protein of claim 21 or the antibody of claim 22, and optionally a nucleoside or nucleotide analog.

29. A method for identifying an inhibitor of a nucleoside or nucleotide kinase comprising the steps of:

(a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a compound to be screened under conditions that permit binding of said compound to the nucleoside or nucleotide kinase, and

(b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the absence or decrease of the signal is indicative for an inhibitor of a nucleoside or nucleotide kinase.

30. A method for identifying a nucleoside or nucleotide based prodrug comprising the steps of

(a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a nucleoside or nucleotide analog to be screened under conditions that permit kinase activity of said polypeptide, and

(b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the presence of a signal is indicative for a putative prodrug.

31. A method for the production of a pharmaceutical composition comprising the steps of

(a) contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a compound to be screened under conditions that permit binding of said compound to the nucleoside or nucleotide kinase, and

(b) detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the absence or decrease of the signal is indicative for an inhibitor of a nucleoside or nucleotide kinase, or

(a') contacting the polypeptide of claim 21 or a cell expressing said polypeptide in the presence of components capable of providing a detectable signal in response to kinase activity, with a nucleoside or nucleotide analog to be screened under conditions that permit kinase activity of said polypeptide, and

(b') detecting presence or absence of a signal generated from the kinase activity of the polypeptide, wherein the presence of a signal is indicative for a putative prodrug; and

(c) formulating the inhibitor identified in step (b) or the nucleoside or nucleotide analog identified in step (b') in a pharmaceutically acceptable form.

32. Use of

(a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide,

(b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17; and/or

(c) the nucleoside or nucleotide analog identified in the method of claim 30

for the preparation of a pharmaceutical composition for the activation of nucleoside or nucleotide analogs or nucleoside or nucleotide based prodrugs and/or for the treatment of viral infections and/or diseases or cancer.

33. The use of claim 32, wherein said activation results in a cytotoxic nucleoside or nucleotide.

34. The use of claim 32 or 33, wherein said viral infection is HIV infection.

35. Use of the inhibitor obtainable by the method of claim 31 for the preparation of a pharmaceutical composition for inhibiting virus replication or for treating cancer.

36. A method for the preparative synthesis of a nucleoside phosphate analog comprising:

(a) using a polynucleotide of claim 14 or as defined in any one of claim 23 to 27 in a noncellular system or in a cell ex vivo, and

(b) formulating the cells modified in step (a) in a pharmaceutically acceptable form.

37. The composition of any one of claims 23 to 27 or the use of any one of claims 32 to 34, wherein said composition is a pharmaceutical composition and further comprises or is designed to be administered with a nucleoside or nucleotide analog, preferably AZT or d4T.

38. Use of

(a) a prokaryotic protein having nucleoside or nucleotide kinase activity for a nucleoside or nucleotide analog or a polynucleotide encoding and capable of expressing said protein *in vivo* or a vector containing said polynucleotide,

(b) the polypeptide of claim 21, the polynucleotide of claim 14 or the vector of any one of claims 15 to 17

for the preparation of nucleoside phosphates or analogs and derivatives thereof.